

The Effect of The Technological Process and Essential Oil of Thyme Against *Salmonella* Enterica Serotype Enteritidis (D) ATCC 13076 in Egg Based Pasta

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Abstract:

Salmonella is a common contaminant of egg and can present a health hazard to consumers. Egg and egg products are an important part of the human diet. Since eggs are used for the production of egg pasta, and due to an insufficient thermal treatment during pasta drying they can be a potential risk for the consumer's health. Different essential oils of herbs can be used in order to reduce potentially present pathogenic microorganisms. The aim of this paper is to describe the impact of the thyme and technological process of production of pasta with eggs on a decrease of the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076. There is not a separate major impact on the concentration of thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076. There is a significant impact of the technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076.

Keywords:

Technological process, Thyme, *Salmonella enterica* serotype Enteritidis (D) ATCC 13076

1. Introduction

Salmonella is a major cause of foodborne disease worldwide, resulting in approximately 93.8 million salmonellosis cases and 155,000 deaths every year (Eng et al., 2015). In general, *Salmonella* outbreaks are frequently associated with the consumption of contaminated fresh fruits and vegetables, live poultry, eggs, raw meat, and meat products (Robertson et al., 2016). Inside the egg, the growth of *Salmonella* is eased by temperature of storage. Eggs should be stored at a constant temperature that should not exceed 20 °C (ACMSF, 1993; Martelli and Davies, 2012). *Salmonella* can grow at 20 °C in the egg albumen, while it is unable to grow at temperatures less than 10 °C. If *Salmonella* reaches the egg yolk, it can grow rapidly, even at room temperature (25 °C) (Martelli and Davies, 2012; Gantois et al., 2009). Humans are most frequently intoxicated with *salmonellae* after they have consumed raw and

undercooked eggs (Martelli and Davies, 2012). During the production of the egg-based pasta drying on the temperature of 46 °C is performed.

Since *Salmonella* Enteritidis can survive even higher temperatures (Blackburn et al., 1997) there is a potential risk of the contamination of the final product. So, additional treatment can be performed in order to ensure elimination of *Salmonella enterica* serotype Enteritidis in egg-based pasta. Antibacterial activity of seven essential oils: cinnamon leaf oil (*Cinnamomum zeylanicum*), garlic oil (*Allium sativum* onion oil (*Allium cepa*), white thyme oil (*Thymus vulgaris*), oregano oil (*Thymus capitatus*), basil oil (*Ocimum basilicum*) and clove bud oil (*Eugenia caryophyllata*) was evaluated against two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella* Enteritidis ATCC 13076) using two preliminary methods: agar disc diffusion method and disc volatilization method. Results showed that all seven essential oils presented antibacterial activity against all the test strains in direct contact method. On the other hand, only two EOs presented significant antibacterial effect through volatilization method against test bacteria. Oregano oil, clove bud oil and white thyme oil showed maximum activity against all the bacteria tested in direct contact method, having a greater inhibition diameter than the reference control, Dobre et al., 2011.

Essential oil of clove, cinnamon, bay and thyme were tested against *Listeria monocytogenes* and *Salmonella* Enteritidis in soft cheese; clove oil was found more effective against *Salmonella* Enteritidis in full fat cheese than in cheese slurry. The most dominant of all identified compounds of thyme EO were thymol (50.48%), followed by p-cymene (24.79%), linalool (4.69%), γ -terpinene (4.14%) and 1,8-cineole (4.35%). Essential oils of thyme exhibited excellent antibacterial activity against *Escherichia coli* and *Salmonella* supposed in test, (Boskovic et al., 2015). *Salmonella* Enteritidis in various foods can be reduced by the use of essential oils of lemongrass, cinnamon leaf, geraniol, thyme, oregano, clove bud, allspice, bay leaf, palmarosa and marjoram oils (Duan and Zhao, 2009; Raybaudi-Massilia et al., 2006).

The results of the study of Ahmadi et al., 2015, show that *Thymus kotschyanus* essential oils have inhibitory effect respectively on yeast, Gram-positive and Gram-negative pathogenic bacteria (yeast > Gram-positive bacteria > Gram-negative bacteria).

The aim of this research is the determination of different concentration of thyme and individual stages of the technological process of production pasta with eggs (mixing, extrusion, drying of pasta, cooling, packaging, storage and distribution) against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076.

2. Materials and Methods

2.1. Egg-based pasta technology and the sampling procedure

Egg-based pasta was made by the following recipe: 10 kg wheat grits and 2 kg wheat flour, 24 eggs, 3.2 – 3.4 L water and 0.010 kg β -carotene. Ingredients were mixed and 25 g of the dough was inoculated with 0.1 mL of the suspension of the investigated species of bacteria with the initial number of bacteria 10^9 CFU/g. After the inoculation, different amounts of sweet basil essential oil (Fitofarm, Skopje, Republic of Macedonia) were added to the prepared dough, at the final concentrations of 1%, 2.5% and 5%. Pasta was formed by extrusion and then dried in the chamber at

the temperature of 46 °C and relative humidity of 80 % for 9 h. Afterwards pasta was cooled at the room temperature for 15 min and packed into PE bags. Samples of the pasta with and without the addition of oils were collected during following production stages: dough making, dough extrusion, drying of pasta, pasta cooling, pasta packaging.

2.2. Microorganisms

The antimicrobial activity of the oils was investigated with following bacteria *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 from the MicroBioLogics, Ins. Joins ATCC Proficiency Standard Program, Minesota, USA.

2.3. Enumeration of bacteria

The determination of the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 was performed according to the Methods of carrying out microbiological analysis and super-analysis of food. The quantity of 25g of pasta was mixed with 225 mL of selenite broth (Torlak, Belgrade, Serbia) and incubated for 24 h at 37°C. Inoculation was carried out by spreading of 0.1 mL of the appropriate dilution on the surface of SS agar (Torlak, Belgrade, Serbia) plates. The enumeration of bacteria was performed after the incubation on 37 °C during 24 hours.

2.4. Statistical Analysis

Data were subjected to analysis using MS Office Excel and the computer programme SPSS 17. Two-factorial experiment showed the significance of differences in means between control and concentration of 1%, 2,5% i 5% of thyme and technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta.

3. Results and Discussion

In the EU, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the serovars most frequently associated with human illness (Guard-Petter, 2001). Human *Salmonella enterica* serotype Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat (EFSA, 2013). Salmonellae are readily destroyed by heat pasteurization of foods at high a_w . As the a_w is reduction with addition of solutes or by removal of water, heat resistance increases markedly. In foods such chocolate, several seconds on 105 °C may be required to reduce *Salmonella* counts by 1 log CFU/g. There is a high probability of infections at doses of $>10^5$ cells but in foods containing high levels of fat and/or protein such as chocolate, salami and cheddar cheese infection can result from ingesting as few as <10 -100 cells (Teunis *et al.*, 2010). In the EU in 2004-2009 was 8 outbreaks with pasta (25% with *Salmonella* spp.), (EFSA, 2012). To minimize the potential risk of salmonellosis due to the consumption of egg and egg products, good manufacturing and handling practices in production of pasta with eggs should always be observed. Initial populations of 430-930 and 1.5-24 cells of *Salmonella* /100 g pasta (MPN Most Probable Number) of pasta (12% moisture) decreased to 0.4-23 and <0.3 -1.5 cells/100g respectively, during storage at room temperature for 360 days (Rayman, *et al.*, 1979). In order to investigate possible effect of essential oil of thyme to the

reduction of the growth of *Salmonella enterica* serotype Enteritidis in egg-based pasta the number of bacteria was determined during different stages of the production process. Pasta was inoculated with *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 with the initial number of bacteria of 10^9 CFU/g and different concentrations of essential oil were added. After extrusion, the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 was decreased by 0.53, 1.0, and 1.53 log CFU / g of pasta for concentration of thyme 1%, 2.5% and 5%. After drying the pasta at 46 °C, humidity of 80% for a period of 9 hours and the inhibition of different concentrations of essential oils of thyme seeded on SS agar were grown colonies, which indicates that the conditions of drying pasta and thyme presence of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 were not rigorous. Addition of 5% of thyme oil lead to the reduction of up to 1.29 log units which was observed during drying. Upon further technological process of cooling the pasta was followed for a period of 15 minutes. After cooling, there is also a pathogen of egg pasta. After phase packaging pasta in plastic bags, the pathogen was also the pasta. Figure 1 shows survival of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in pasta with thyme during technological process.

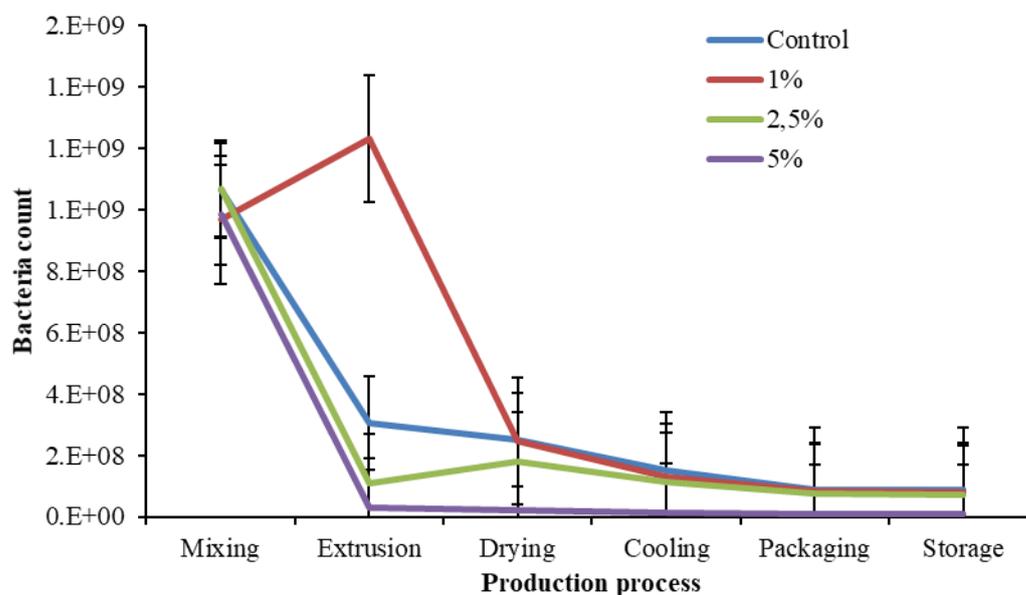


Figure 1. Survival of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in the pasta with essential oil of thyme.

Essential oils of spices and herbs have been used as food additives, as flavoring agent and as natural food preservatives since ancient times. A number of spices have antimicrobial activity against different types of microorganisms (Škrinjar and Nemet, 2009; Tajkarami *et al.*, 2010). Comparison of the effect of different concentrations of the thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta showed that the mean difference was significant at the 0,05 level between the thyme concentration of 1% and 5%. For a concentration of 1% of thyme, and thyme concentration of 5%, $p=0.024$ (Table 1).

Table 1. Impact of different concentration of thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta.

Pairwise Comparisons						
Dependent Variable: <i>Salmonella enterica</i> serotype Enteritidis (D) ATCC 13076						
(I) Concentration of thyme	(J) Concentration of thyme	Mean Difference (I-J)	Std. Error	Sig. ^b	95% Confidence Interval for Difference ^b	
					Lower Bound	Upper Bound
Control	1%	-131244444,444	119289832,623	,277	-371092728,147	108603839,258
	2,5%	54988888,889	119289832,623	,647	-184859394,813	294837172,591
	5%	147572777,778	119289832,623	,222	-92275505,924	387421061,480
1% thyme	Control	131244444,444	119289832,623	,277	-108603839,258	371092728,147
	2,5%	186233333,333	119289832,623	,125	-53614950,369	426081617,035
	5%	278817222,222*	119289832,623	,024	38968938,520	518665505,924
2,5% thyme	Control	-54988888,889	119289832,623	,647	-294837172,591	184859394,813
	1%	-186233333,333	119289832,623	,125	-426081617,035	53614950,369
	5%	92583888,889	119289832,623	,441	-147264394,813	332432172,591
5% thyme	Control	-147572777,778	119289832,623	,222	-387421061,480	92275505,924
	1%	-278817222,222*	119289832,623	,024	-518665505,924	-38968938,520
	2,5%	-92583888,889	119289832,623	,441	-332432172,591	147264394,813

Based on estimated marginal means

*. The mean difference is significant at the, 05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Essential oils of cinnamon, oregano and mustard are efficient in the reduction of the number *Salmonella* in beef (Turgis et al., 2008), while a concentration of 2 µL/mL cinnamon, geranium, lemongrass and palmarosa oils decreased the number of *Salmonella enterica* serotype Enteritidis in fruit juice (Raybaudi-Massilia et al., 2006). Thanissery and Smith (2014) found that a combination of thyme and orange essential oils (TOC) inhibited the growth of both *Salmonella* and *Campylobacter* when used at a level of 0.5%. Essential oils of *Thymus vulgaris*, *Mentha piperita*, *Rosmarinus officinalis*, showed strong antimicrobial activity (both bacteriostatic and bacteriocidal effect) against *Salmonella* Enteritidis and *Escherichia coli* in concentrations ranged from 0,125 to 2% (v/v) (Nicuale et al., 2009).

Table 2. Impact of the technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta.

Pairwise Comparisons						
Dependent Variable: <i>Salmonella enterica</i> serotype Enteritidis (D) ATCC13076						
(I) Proses	(J) Proses	Mean Difference (I-J)	Std. Error	Sig. ^b	95% Confidence Interval for Difference ^b	
					Lower Bound	Upper Bound
Mixing	Ekstrusion	600808333,333*	146099610,714	,000	307055377,957	894561288,710
	Drying	845808333,333*	146099610,714	,000	552055377,957	1139561288,710
	Cooling	918241666,667*	146099610,714	,000	624488711,290	1211994622,043
	Packaging	955916666,667*	146099610,714	,000	662163711,290	1249669622,043
	Storage	957134166,667*	146099610,714	,000	663381211,290	1250887122,043
Ekstrusion	Mixing	-600808333,333*	146099610,714	,000	-894561288,710	-307055377,957
	Drying	245000000,000	146099610,714	,100	-48752955,376	538752955,376
	Cooling	317433333,333*	146099610,714	,035	23680377,957	611186288,710
	Packaging	355108333,333*	146099610,714	,019	61355377,957	648861288,710
	Storage	356325833,333*	146099610,714	,018	62572877,957	650078788,710
Drying	Mixing	-845808333,333*	146099610,714	,000	1139561288,710	-552055377,957
	Ekstrusion	-245000000,000	146099610,714	,100	-538752955,376	48752955,376
	Cooling	72433333,333	146099610,714	,622	-221319622,043	366186288,710
	Packaging	110108333,333	146099610,714	,455	-183644622,043	403861288,710
	Storage	111325833,333	146099610,714	,450	-182427122,043	405078788,710
Cooling	Mixing	-918241666,667*	146099610,714	,000	1211994622,043	-624488711,290
	Ekstrusion	-317433333,333*	146099610,714	,035	-611186288,710	-23680377,957
	Drying	-72433333,333	146099610,714	,622	-366186288,710	221319622,043
	Packaging	37675000,000	146099610,714	,798	-256077955,376	331427955,376
	Storage	38892500,000	146099610,714	,791	-254860455,376	332645455,376
Packaging	Mixing	-955916666,667*	146099610,714	,000	1249669622,043	-662163711,290
	Ekstrusion	-355108333,333*	146099610,714	,019	-648861288,710	-61355377,957
	Drying	-110108333,333	146099610,714	,455	-403861288,710	183644622,043
	Cooling	-37675000,000	146099610,714	,798	-331427955,376	256077955,376
	Storage	1217500,000	146099610,714	,993	-292535455,376	294970455,376
Storage	Mixing	-957134166,667*	146099610,714	,000	1250887122,043	-663381211,290
	Ekstrusion	-356325833,333*	146099610,714	,018	-650078788,710	-62572877,957
	Drying	-111325833,333	146099610,714	,450	-405078788,710	182427122,043
	Cooling	-38892500,000	146099610,714	,791	-332645455,376	254860455,376
	Packaging	-1217500,000	146099610,714	,993	-294970455,376	292535455,376

Based on estimated marginal means

*. The mean difference is significant at the, 05 level.
 b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Salmonella can survive in date paste and on cranberries and raisins stored at 4 °C for at least 8 months but for less than 18, 7, and 7 weeks, respectively, at 25 °C (Beuchat et al., 2014). Comparison within the group (different process: mixing, extrusion, drying, cooling, packaging, and storage and distribution) in the two-factorial experiment showed that there was statistically significant at $p < 0.05$ (Table 2) between the mixing and all other processes ($p=0.000$), between extrusion and cooling ($p=0.035$), extrusion and packaging ($p=0.019$), extrusion, and storage and distribution ($p=0.0018$), drying and mixing ($p=0.000$), cooling and mixing ($p=0.000$) and extrusion ($p=0.035$), packaging with mixing and extrusion, and storage and distribution with mixing and extrusion.

Table 3. Impact of the thyme and technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta.

Tests of Between-Subjects Effects					
Dependent Variable: <i>Salmonella enterica</i> serotype Enteritidis (D) ATCC13076					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1133484793819861 8000,000 ^a	23	49281947557385299 0,000	3,848	,000
Intercept	6786597276501412 900,000	1	67865972765014129 00,000	52,991	,000
Concentration of thyme	7335912652819457 30,000	3	24453042176064857 6,000	1,909	,141
Proces	8391937945340273 700,000	5	16783875890680547 80,000	13,105	,000
Concentration * Proces	2209318727576388 860,000	15	14728791517175926 4,000	1,150	,341
Error	6147387720199995 400,000	48	12807057750416657 6,000		
Total	2426883293489999 0000,000	72			
Corrected Total	1748223565839861 4000,000	71			

a. R Squared = ,648 (Adjusted R Squared = ,480)

There is not significant differences in the effects of the concentration of thyme and technological process against *Salmonella enterica* serotype Enteritidis ATCC 13076 in the production of pasta ($p=0.341$). There is not a separate major impact on the concentration of thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 ($p=0.141$). There is a significant impact of the technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 ($p = 0.000$) (Table 3).

4. Conclusions

Comparison of the effect of different concentrations of the thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta showed that the mean difference was significant at the 0,05 level between the thyme concentration of 1% and 5%. For a concentration of 1% of thyme, and thyme concentration of 5%. There is not a separate major impact on the concentration of thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076. There is a significant impact of the technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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